

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## The Effect of the Detergent Micelles Type on the Tetrapeptide NAc-SFVG-OMe Conformational Structure: NMR Studies in Solution.

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### ABSTRACT

Process determination of short peptides binding on the cell surface has major implications in better understanding the molecular recognition of cell surfaces. As such methods as on-cell nuclear magnetic resonance (NMR) spectroscopy are very difficult, a large number of membrane mimetic systems such as bilayers, bicelles and detergent micelles use. Micelles are the most frequently used membrane mimetics for the structure determination of peptides and proteins by solution NMR. Anionic detergents such as SDS can be more denaturing than the other types, non-ionic micelles being the mildest. Zwitterionic detergent micelles such as DPC are used to mimic eukaryote membranes while the negatively charged SDS micelles would resemble bacterial membranes. Unfortunately, no rules apply when searching for the right detergent. In present paper we studied the effect of the detergent micelles (sodium dodecyl sulfate (SDS) and dodecylphosphocholine (DPC)) on the tetrapeptide SFVG conformational structure. Was shown that the peptide backbone structure is the same in both types of micelles but the sidechain orientation of nonpolar aromatic (Phenylalanine), aliphatic (Glycine) and polar uncharged (Serine) groups are different

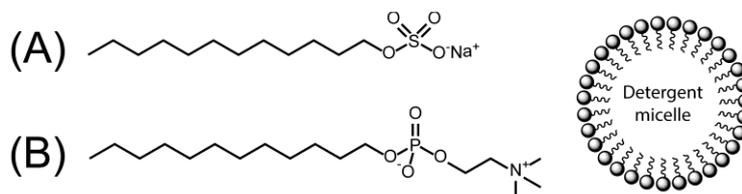
**Keywords:** membrane mimetics, detergent, NMR, protein structure, oligopeptides

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## INTRODUCTION

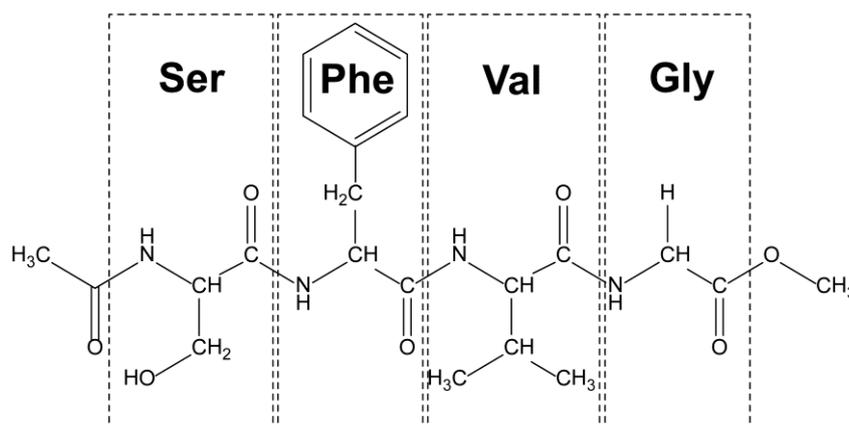
Nuclear Overhauser Effect (NOE) spectroscopy is a widely used technique which allows to obtain information about the 3-dimensional structure of the molecule under consideration in solution. The intensity of the NOESY cross-peak depends on the molecular correlation time (or the inverse of the rate of molecular tumbling), which is in large part determined by the molecular weight. For small molecules, the NOESY cross-peak generally weaker and may be zero for medium-sized molecules (MW range 700 – 1200) and so it is not possible to obtain any information on inter-proton distances using this technique. It is quite common for small peptides consisting of only a few residues. But if such peptides will be dissolved with some membrane mimetic and it will bind on its surface then the molecular weight of the total complex increase and also as the NOE cross-peaks intensity [1, 2].

Detergents are widely used in biochemistry, cell biology, structural biology or molecular biology. Cell lysis, protein solubilization, protein crystallization or reduction of background staining in blotting experiments are just a few of numerous applications. Process determination of short peptides binding on the cell surface has major implications in better understanding the molecular recognition of cell surfaces. As such methods as on-cell nuclear magnetic resonance (NMR) spectroscopy are very difficult, a large number of membrane mimetic systems such as bilayers, bicelles and detergent micelles use. Micelles are the most frequently used membrane mimetics for the structure determination of peptides and proteins by solution NMR [3]. These aggregates are formed by self-assembly of amphiphilic molecules in solution in order to minimize the contact of their hydrophobic tails with the aqueous environment [4]. Sodium dodecyl sulfate (SDS) and dodecylphosphocholine (DPC) micelles are the most commonly used for structure determination of membrane proteins and peptides (Fig.1). Anionic detergents such as SDS can be more denaturing than the other types, non-ionic micelles being the mildest [5, 6]. Zwitterionic detergent micelles such as DPC are used to mimic eukaryote membranes while the negatively charged SDS micelles would resemble bacterial membranes [7, 8]. Unfortunately, no rules apply when searching for the right detergent.



**Figure 1: Molecular structure of commonly used surfactants for the preparation of membrane mimetics (A) sodium dodecylsulfate (SDS); (B) n-dodecylphosphocholine (DPC)**

In order to understand how detergent micelle type will effect on the peptide spatial structure, in present paper we studied tetrapeptide with protecting groups on both ends (Nac-SFVG-OMe) which contains residues with polar uncharged groups (Serine); nonpolar aromatic groups (Phenylalanine); nonpolar aliphatic groups (Valine and Glycine) (Fig.2).



**Figure 2: Chemical structure of tetrapeptide NAc-SFVG-OMe**

This peptide is a very good and simple model due to the relative simplicity of its  $^1\text{H}$  NMR spectrum in water; it contains aromatic and aliphatic side-chains, the simple amino acid glycine and an additional polar group in serine; there are no overlapping signals in NOE because of short amino acid sequence. Also it was well studied previously by residual dipolar couplings (RDC) analysis, circular dichroism (CD), solid state NMR and high-resolution NMR spectroscopy in water, TFE, and SDS micelles [9, 10]. The peptide does not possess any known preferential conformation either in water or in TFE [9]. In SDS micelle based on the hydrophobic consideration was obtained structure of the “tetrapeptide NAc-Ser-Phe-Val-Gly-OMe - SDS micelle” complex in water solution [11]. In present paper we determined spatial structure of the tetrapeptide in solution with zwitterionic DPC micelles and compared it with determined previously one in anionic SDS micelles.

## EXPERIMENTAL

### Sample preparation

The tetrapeptide NAc-Ser-Phe-Val-Gly-OMe was obtained as a gift from Professor S. Berger, Leipzig University. The peptide (4 mg) was solubilized in an aqueous solution (90%  $\text{H}_2\text{O}$  + 10%  $^2\text{H}_2\text{O}$ , 500  $\mu\text{l}$ ) containing 20 mg perdeuterated DPC. Perdeuterated  $\text{d}_{38}$  DPC (98%  $^2\text{H}$ ) and TSP- $\text{d}_4$  were purchased from Aldrich.

### NMR spectroscopy and spatial structure calculation

All data were acquired at 500 MHz (Bruker Avance II) NMR spectrometer at a probe temperature 293 K. The sample temperature was set to temperature 298 K.

Two-dimensional (2D) experiments (DQF-COSY, TOCSY, and NOESY) spectra were acquired in the phase-sensitive mode using the States-TPPI method and using a time domain data size of 512  $t_1$  x 4096  $t_2$  complex points and 32 transients per complex  $t_1$  increments. The water resonance was suppressed by “3-9-19” pulse sequence with gradients using flip-back pulse in COSY experiments [12, 13] and using excitation sculpting with gradients in TOCSY and NOESY experiments [14]. TOCSY spectra were obtained with a mixing time of 80 ms and NOESY spectra with a mixing time of 400, 500 ms.

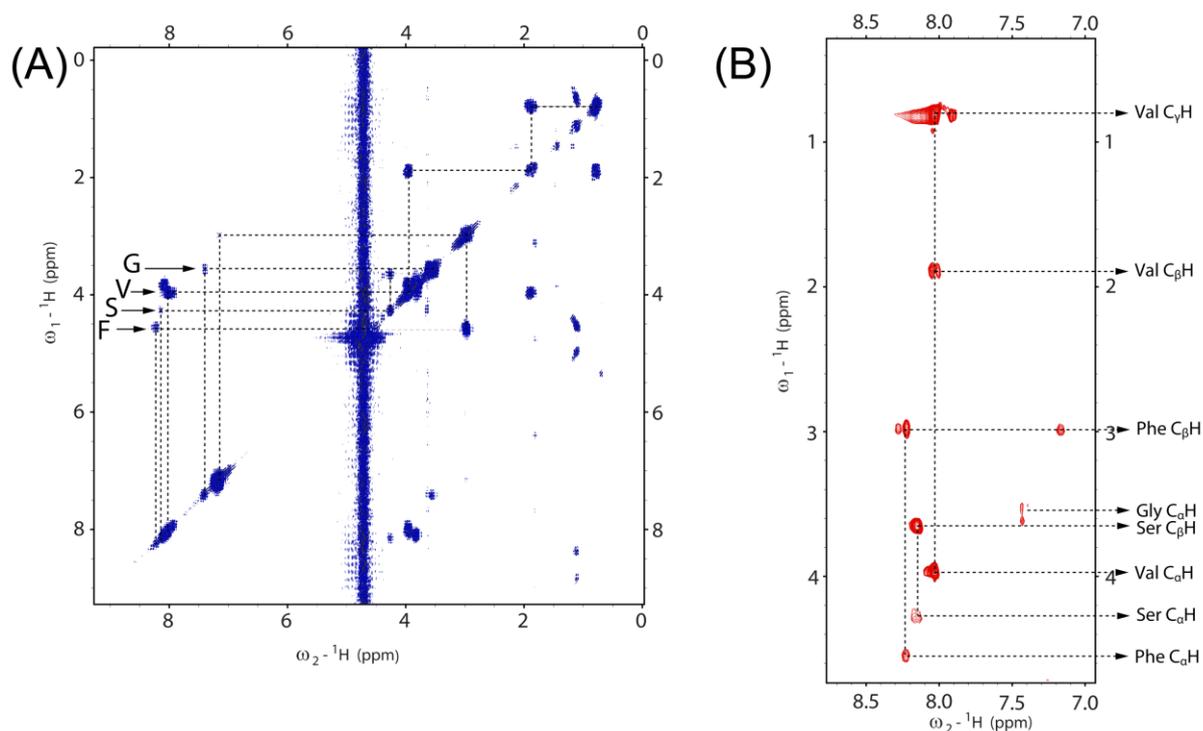
Spectra were processed by NMRPipe [15] and analyzed using SPARKY. Sequence-specific backbone  $^1\text{H}$  resonance assignments and side-chain assignments for all residues were obtained using a combination of 2D COSY and TOCSY experiments.

Inter-proton distances obtained from analysis of intensities of cross-peaks from NMR NOESY spectra were used as the primary data for the calculations by the molecular dynamics method. Following structural calculations, the ensemble of structures was subjected to restrained molecular dynamics using the Xplor-NIH [16]. A total of 500 structures were calculated and 20 with minimal energy were chosen. None of the 20 structures had any violated nuclear Overhauser effect (NOE) distances. Individual structures were minimized, heated to 1000 K for 6000 steps, cooled in 100 K increments to 50 K, each with 3000 steps, and finally minimized with 1000 steps of the steepest descent, followed by 1000 steps of conjugate gradient minimization. Starting with a family of 1000 structures, approximately 200 were subjected to subsequent molecular dynamics calculations and, finally, the 20 lowest energy structures were retained. The tetrapeptide structures were visualized with CHIMERA [17].

## RESULTS AND DISCUSSION

**Table 1:**  $^1\text{H}$  chemical shifts in ppm measured in water for tetrapeptide NAc-SFVG-OMe in the presence of perdeuterated DPC micelles at 298 K.

Residue	$J^{(\text{HNH}\alpha)}$	NH	$\text{C}_\alpha\text{H}$	$\text{C}_\beta\text{H}$	$\text{C}_\gamma\text{H}$	$\text{C}_\delta\text{H}$	$\text{C}_\epsilon\text{H}$
		Ser	16 Hz	8.15	4.27	3.64	-
Phe	12 Hz	8.24	4.62	2.98	-	7.16	-
Val	15 Hz	8.03	3.95	1.88	0.8	-	-
Gly	-	7.39	3.55	-	-	-	-



**Figure 3: Fingerprint regions of 2D NMR spectra: (A)  ${}^1\text{H}$ - ${}^1\text{H}$  COSY; (B)  ${}^1\text{H}$ - ${}^1\text{H}$  TOCSY acquired at 500 MHz of tetrapeptide NAc-SFVG-OME in a solution of  $\text{H}_2\text{O}+\text{D}_2\text{O}$  with DPC micelles, 298 K.**

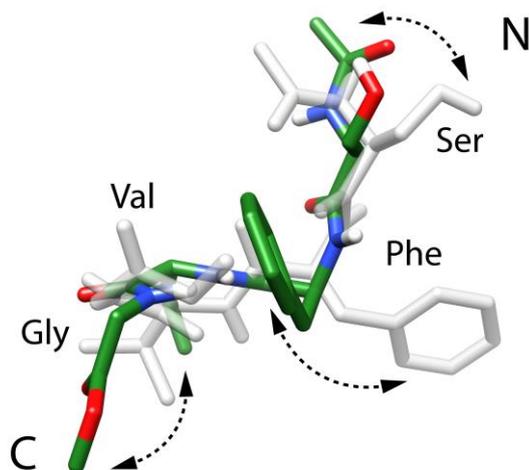
Proton chemical shift assignments of tetrapeptide NAc-SFVG-OME in DPC micelles were obtained using standard methods of protein NMR spectroscopy by 2D NMR  ${}^1\text{H}$ - ${}^1\text{H}$  COSY and  ${}^1\text{H}$ - ${}^1\text{H}$  TOCSY (Table 1) [18-20]. J-coupling  $\text{H}_\text{N}$ - $\text{C}_\alpha\text{H}$  constants were obtained from 2D  ${}^1\text{H}$ - ${}^1\text{H}$  DQF-COSY spectrum (Table 1.)

Fig.3 shows the fingerprint region of the  ${}^1\text{H}$ - ${}^1\text{H}$  COSY and  ${}^1\text{H}$ - ${}^1\text{H}$  TOCSY spectra of tetrapeptide NAc-SFVG-OME at 298 K in  $\text{H}_2\text{O}$  solution and in the presence of DPC. As expected, four well resolved spin system were observed in proton spectra. J-coupling constants and 10 interproton NOE distance constraints (see Table.2) which were determined from  ${}^1\text{H}$ - ${}^1\text{H}$  NOESY spectra were used as the primary data for the tetrapeptide NAc-SFVG-OME structure calculations by the molecular dynamics method.

**Table 2: Experimental interproton distances for the heptapeptide NAc-Lys-Leu-Val-Phe-Phe-Ala-Glu-NH<sub>2</sub> in a solution of  $\text{H}_2\text{O}+\text{D}_2\text{O}$  with DPC micelles.**

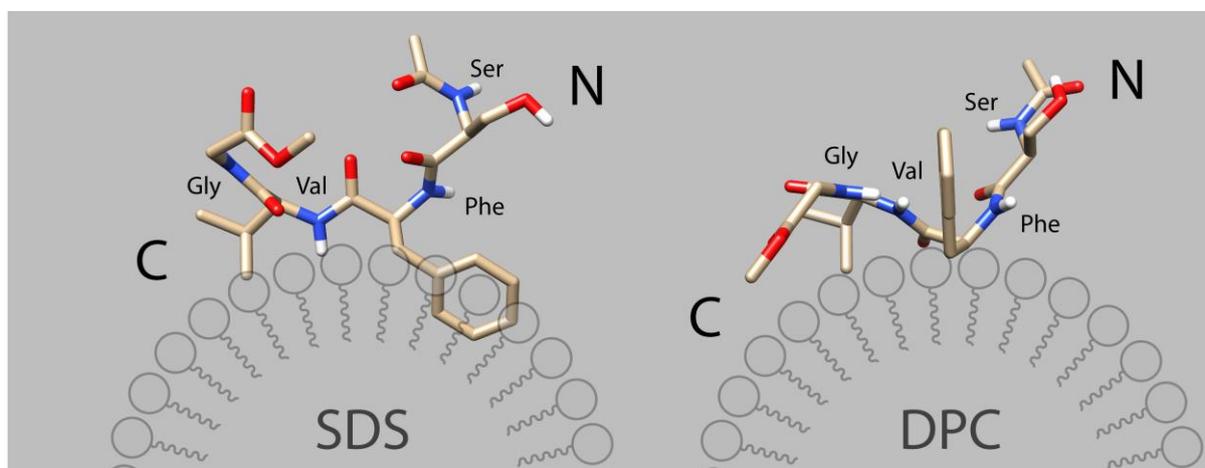
Interacting protons	Distance $r$ , Å, NOESY	Distance $r$ , Å, XPLOR
Phe( $\text{C}_\alpha\text{H}$ )-Phe( $\text{C}_\beta\text{H}$ )	$2.6\pm 0.52$	2.33
Val( $\text{C}_\alpha\text{H}$ )-Val( $\text{C}_\gamma\text{H}$ )	$2.4\pm 0.48$	2.45
Val( $\text{C}_\alpha\text{H}$ )-Val( $\text{C}_\beta\text{H}$ )	$2.5\pm 0.50$	2.40
Val( $\text{C}_\beta\text{H}$ )-Val( $\text{C}_\gamma\text{H}$ )	$3.0\pm 0.60$	2.68
Val( $\text{C}_\beta\text{H}$ )-Val( $\text{H}_\text{N}$ )	$3.5\pm 0.70$	3.73
Phe( $\text{C}_\alpha\text{H}$ )-Phe( $\text{C}_\delta\text{H}$ )	$4.0\pm 0.8$	4.70
Phe( $\text{C}_\beta\text{H}$ )-Phe( $\text{C}_\delta\text{H}$ )	$2.5\pm 0.50$	2.98
Val( $\text{H}_\text{N}$ )-Val( $\text{C}_\alpha\text{H}$ )	$2.7\pm 0.54$	2.63
Phe( $\text{C}_\alpha\text{H}$ )-Val( $\text{H}_\text{N}$ )	$2.6\pm 0.52$	2.71
Gly( $\text{H}_\text{N}$ )-Gly( $\text{C}_\alpha\text{H}$ )	$2.4\pm 0.48$	2.82

500 structures were calculated and 20 with minimal energy were chosen. Stereo view of a structure with minimal energy is shown in Figure 4.



**Figure 4: Cartoon of tetrapeptide NAc-SFVG-OMe in a solution with detergent micelles (DPC (green), SDS (white)) [11] stereo view, representing the NMR structure.**

Comparing obtained structure of tetrapeptide NAc-SFVG-OMe in H<sub>2</sub>O solution in the presence of DPC with the same one in SDS micelles we can conclude that the peptide backbone structure is the same in both types of micelles, but the sidechain orientation of nonpolar aromatic (Phenylalanine), aliphatic (Glycine) and polar uncharged (Serine) groups are different. Due to that fact that a membrane surface with positive curvature allows the hydrophobic amino acid residues to be buried while the charged residues remain solvated in water here we speculate that as in case with SDS micelles [11], in DPC micelles hydrophobic area of peptide (Valine residue) is oriented toward the micelle [21-23]. Proposed conformation of tetrapeptide NAc-SFVG-OMe in a solution with detergent DPC and SDS micelles is present in Figure 5.



**Figure 5: Proposed structures for tetrapeptide NAc-SFVG-OMe in a solution with detergent DPC and SDS micelles.**

Generally our data are in a good agreement with backbone tetrapeptide NAc-SFVG-OMe structure in SDS micelles (RMSD between two structures 0.8Å) [11], and the conformational changes in peptide sidechains orientation could be explained by the effect of the different type of the anionic (SDS) and zwitterionic(DPC) detergent micelles surface. This fact may have important biological significance and requires further structural studies of larger proteins.

### CONCLUSION

In present paper we studied the effect of the detergent micelles (sodium dodecyl sulfate (SDS) and dodecylphosphocholine (DPC)) on the tetrapeptide NAc-SFVG-OMe conformational structure. Was shown that the peptide backbone structure is the same in both types of micelles but the sidechain orientation of nonpolar aromatic (Phenylalanine), aliphatic (Glycine) and polar uncharged (Serine) groups are different. The

conformational changes in peptide sidechains orientation could be explained by the effect of the different type of the anionic (SDS) and zwitterionic (DPC) detergent micelles surface. This fact may have important biological significance and requires further structural studies of larger proteins.

#### ACKNOWLEDGEMENTS

The work is performed accordingly to the Russian Government Program of Competitive Growth of Kazan Federal University; by the subsidy allocated to Kazan Federal University for the project part of the state assignment in the sphere of scientific activities and also supported by Russian Foundation for Basic Research (grant 14-04-31029 mol\_a).

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